

LAXATIVE AND ANTIMICROBIAL ACTIVITY OF *SENNA SPECTABILIS* (CAESALPINIACEAE)

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ABSTRACT.- Inhabitants that practice folk medicine in the Andean Region of Venezuela use preparations of *Senna spectabilis* (Caesalpinaceae) for several remedies including natural laxatives, but biological activity of this botanical species is still undocumented. We characterized foliar material from *Senna spectabilis* (var. *spectabilis*) chemically and biologically. Crude extracts from leaves were prepared using organic solvents with increasing polarity, and assayed *in-vivo* for laxative activity on albino, Balbc mice. Additionally, presence of *in-vitro* antimicrobial activity was established against bacterial strains of *Escherichia coli*, *Staphylococcus aureus* and *Bacillus* sp. Ethyl acetate (water soluble form) and methanol extracts showed moderate laxative activity. We characterized compounds isolated from ether extracts as carboxyl acids and compounds from methanol extracts as anthraquinones. Antibacterial activity of *Senna spectabilis* extracts (except ether and benzene) on *Bacillus* sp. colony growth is comparable to that of commercial discs, and indicates an interesting antimicrobial potential against other sporogenous strains of clinical and industrial importance. *Received:* 08 December 1999, *accepted:* 28 April 2000.

Key words: Antimicrobial activity, Caesalpinaceae, folk medicine, laxative activity, *Senna spectabilis*, Venezuela.

ACTIVIDAD LAXATIVA Y ANTIMICROBIANA DE *SENNA SPECTABILIS* (CAESALPINIACEAE)

RESUMEN.- En la Región Andina de los Andes de Venezuela, los habitantes que practican la medicina folklórica utilizan preparaciones de *Senna spectabilis* (Caesalpinaceae) en varias aplicaciones, la más común es la laxativa. Sin embargo, la actividad biológica de esta especie botánica no está documentada en la literatura. En este estudio, se caracterizó la materia foliar de *Senna spectabilis* (var. *spectabilis*) químicamente y biológicamente. Se obtuvieron extractos crudos a partir de las hojas utilizando solventes orgánicos en orden creciente de polaridad, los cuales fueron ensayados *in-vivo* en ratones albinos Balbc, para establecer la presencia de actividad laxativa. Adicionalmente, se estableció la presencia de actividad antimicrobiana *in-vitro* sobre cepas bacterianas de *Escherichia coli*, *Staphylococcus aureus* y *Bacillus* sp. Los resultados obtenidos con la fracción ácuea proveniente del extracto acetato de etilo y el extracto metanólico demostraron un efecto laxante moderado. Los compuestos aislados provenientes de los extractos en éter de petróleo y metanólico fueron caracterizados como un posible ácido carboxílico y un compuesto antraquinónico respectivamente. La actividad antimicrobiana de los extractos de *Senna spectabilis* (excepto éter y benceno) contra *Bacillus* sp. muestra un potencial antimicrobiano contra otras cepas bacterianas de importancia clínica e industrial. *Recibido:* 08 Diciembre 1999, *aceptado:* 28 Abril 2000.

Palabras claves: Actividad antimicrobiana, actividad laxativa, Caesalpinaceae, medicina folklórica, *Senna spectabilis*, Venezuela.

INTRODUCTION

Several species of *Senna*, *Cassia* and *Chamaecrista* exhibit medicinal properties based on the presence of secondary metabolites with biological activity distributed throughout the plants (Joshi *et al.* 1985, Khare *et al.* 1980, Singh 1981, Singh and Singh 1986, Wong *et al.* 1989a, and Wong *et al.* 1989b). In the Andean region of Venezuela (Mérida, Trujillo and Táchira States), botanical

preparations with *Senna spectabilis* (Caesalpinaceae) are commonly used in folk medicine for several remedies, primarily laxative, but the biological activity of this plant species is still undocumented in the literature. In this study, we characterize the laxative inducing activity present in *Senna spectabilis* foliar material. Also, *in vitro* antimicrobial activity of *S. spectabilis* extracts is tested on selected aerobic bacteria to determine the effect on normal gut microflora.

MATERIALS AND METHODS

We collected fresh foliar material (leaves) of *Senna spectabilis* in the lower altitude zone of Trujillo State, Venezuela. Specimens previously identified by personnel of the Botanical Area, Biology Department, Faculty of Sciences, University of Zulia, were paper wrapped and dehydrated in an oven at 40 °C. The material was minced in a mill (Thomas scientific Mod. 3383-L10, screen 40 mesh) and subjected to exhaustive organic solvent extraction in a soxhlet condenser. Petroleum ether, benzene, chloroform ethyl acetate and methanol solvents were used sequentially to obtain extracts. The extracts were concentrated in a rotovapor (Buchi-R110) under reduced pressure and temperature. We used only Fisher or Merck analytical grade reagents.

Extracts were chromatographed on silica-gel (180 g and 350 g) columns of 70-230 mesh (Merck). Isolated compounds were characterized according to qualitative colorimetric reactions, physical properties and spectroscopy (IR and UV). Fusion points (uncorrected) were obtained in a Fisher-Johns (20-300 °C) melting point apparatus. The IR spectrums were obtained in a Perkin-Elmer apparatus, model 1725X with Fourier Transform. The UV spectrum of ethanol-diluted sample solutions was recorded in a Perkin-Elmer model Lambda 3B spectrophotometer. The various chemical studies used for compound characterization included a test for: phenolic oxyhydriles (Domínguez 1973), 2,4-dinitrophenylhydrazine (Cheronis and Entrinkin 1963), sodium hydrosulfite for quinones (Domínguez 1973), Bornträger test for anthraquinones (Albornoz 1980), cyanidine and aluminum trichloride for flavonoids (Albornoz 1980), NaOH and sodium bicarbonate for acid groups (Cheroniz and

Entrinkin 1963), and the Liebermann-Burchard test for sterols and triterpenes (Dominguez 1973).

Potential laxative activity of the crude extracts was assayed *in-vivo* on 30 albino, male Balbc mice, approximately 20 g body mass and 8–10 weeks old. Mice were fed a commercial diet (Purina®) unless otherwise indicated, and water was provided *ad-libitum*.

Extract samples (35 mg) were formulated into a diet ration combined with locally obtained Cheddar cheese to yield palatable pellets circa 4 mm in size. Senokot (Farma S. A.), a commercial natural laxative formula comprising senosides A and B from *Cassia augustifolia*, was used as a positive control. The control dosage was 8 mg, calculated per body mass, according to the manufacturer's indications. The final preparation was formulated and administered as previously indicated. The negative control was fed a cheese-only diet.

We determined extract laxative activity by counting fecal droppings eliminated by the treated animals vs. the controls. Counting (in triplicate) was performed every 2 h, during 24 h following extract administration. The mean number of animal defecations was compared to the control, and a Duncan (Bauer *et al.* 1966) statistical variance test was performed.

We assayed crude extract antimicrobial activity *in-vitro* using the disc technique described by Kirby Bauer (Marcano and Hasegawa 1991), with modifications. A 60 mg aliquot of the extract dilution was placed on the surface of a bacterially inoculated plate seeded for confluent growth. Pure solvents were used as a control. Following incubation at 37 °C for 24 h, the diameter of inhibited bacterial colony growth was measured and accrued. We used the following bacterial strains: *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923 and *Bacillus* sp. Commercial antibiotic discs of Cloramfenicol (K) (30 µg), Gentamycin (G) (10 µg) and Penicillin (P) (10 IU) were used as a positive control and to establish comparable antimicrobial activity. Microorganisms were

cultivated on trypticase soy nutrient broth media (TSB) (Merck 016V-833143) and trypticase soy agar media (TSA, CTS) (Merck 107V-930850). An inoculum corresponding to each strain was previously incubated at 37°C for 24h, and then seeded in tubes containing 4 ml of TSB. From this primary stock, we determined a bacterial cell culture growth curve and cell density. The bacterial cell suspension dilution was seeded onto 15 ml TSA media plates, using an L-rod technique. Prior to antibiotic sensitivity testing, *E. coli*, *S. aureus* and *Bacillus* sp. cell densities were standardized to 3×10^5 , 3×10^4 and 3×10^5 CFU/ml, respectively.

RESULTS

PURIFICATION AND COMPOUND CHEMICAL CHARACTERIZATION

Compound 1.- A 4 g aliquot was obtained from the petroleum ether extraction chromatography. The extract consisted of granular off-white solid crystals, recrystallized with a mixture of petroleum ether: ethyl acetate (2:1). The refined crystals decomposed at 278°C. IR $\nu(\text{max})$ (cm^{-1}) 3515 and 3335 (OH), 2967 and 2875 (C-H alifatics) 1726 (C = O), 1651 (C = C), 1472 (CH_2) and 1207 (C-OH) (Fig. 1). The compound was soluble in NaOH 2.5 N and effervesced over a solution of sodium bicarbonate 1.5 N. Analysis of the IR spectrum data revealed several bands: two at 3515 and 3335 cm^{-1} , corresponding to a free hydroxyl group, and an intense band (1725 cm^{-1}) attributable to C = O bond vibrations. Two intense bands at 2967 and 2875 cm^{-1} corresponded to aliphatic carbon-hydrogen bonds, and another at 1651 cm^{-1} , corresponded to a double carbon-carbon bond. According to the data, the material corresponded to a carboxylic acid. Structural elucidation will require further spectroscopic study.

Compound 2.- A 10g methanol extract from the chromatography column, was diluted with a mixture of petroleum ether: ethyl acetate (1:9). The material was reddish, with a 295 °C melting point, UV λ (max) 227, 258, 286 and 431 nm (Fig. 2). Testing of this compound for flavonoids was negative, while the

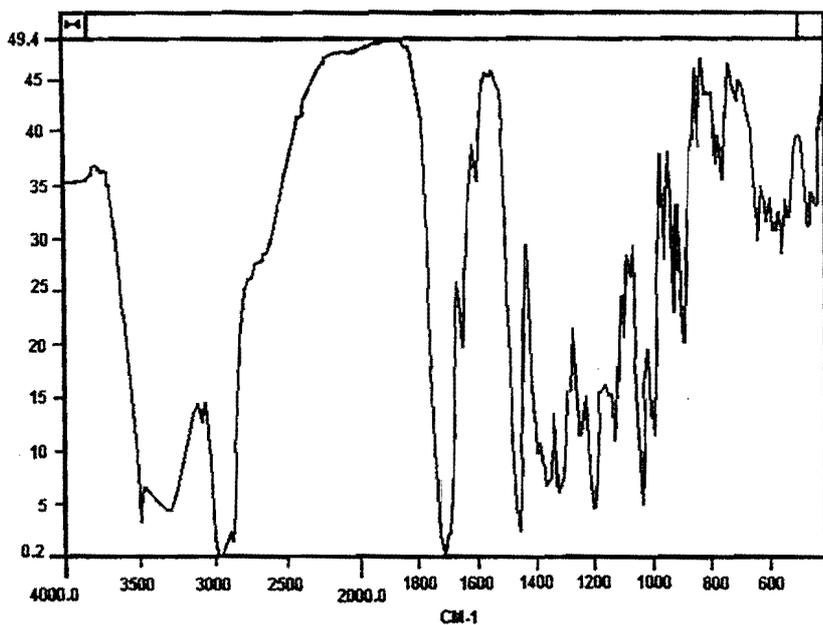


FIGURE 1. IR spectrum of compound 1

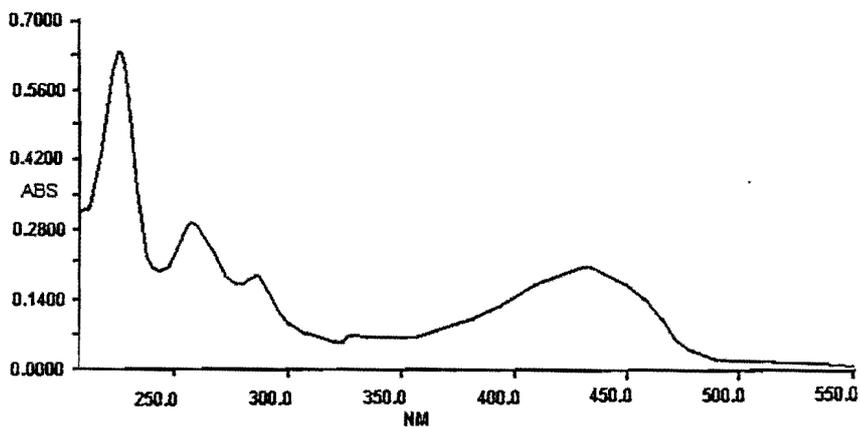


FIGURE 2. UV spectrum of compound 2.

Bornträger and phenolic oxhydrile tests were positive. The four UV absorption bands detected corresponded to an anthraquinone compound with substituted hydroxyl or methoxyl groups on positions 1.8 or 1.5, according to the longest wavelength absorption (Marcano and Hasegawa 1991). Analysis of the results indicates the presence of a dihydroanthraquinone, but detailed spectroscopic study may be needed to elucidate a final structure.

BIOLOGICAL ASSAY

Laxative activity.- The results indicate that the ether, benzene, chloroform extracts, and the water insoluble fraction from the ethyl acetate extract, failed to induce a laxative effect on the study animals (Tables 1 and 2). Duncan test variance determinations ($P<0.05$) indicated a lack of statistical difference between mean numbers of defecations of study animals exposed to the extracts vs. the negative control. However, results obtained with the water-soluble form of ethyl acetate and methanol extracts corresponded to moderate laxative activity, when compared to the positive control (Table 2).

TABLE 1. Mean number of defecations of study animals fed ether, benzene and chloroform extracts (n = 3).

TREATMENT	MEAN NO. DEFECATIONS ±SD	TOTAL DEFECATION (ANOVA)	MEAN DEFECATION/2 H
Positive control (Senokot)	78.7 ±5.0	78.66	6.55
Negative control	54.0 ±1.0	54.00	4.50
Ether extract	53.7 ±10.0	53.66*	4.40*
Benzene extract	57.0 ±4.0	57.00*	4.74*
Chloroform extract	56.3 ±1.7	56.33*	4.70*

*Values not significant ($P<0.05$).

Antimicrobial activity.- Extract antibacterial action against *E. coli* and *S. aureus* (Table 3) was not significant, except for the water soluble fraction obtained from ethyl acetate extract, where colony growth halo size was comparable to that of gentamycin commercial

TABLE 2. Mean number of defecations of study animals fed ethyl acetate derived extracts (n = 3).

TREATMENT	MEAN NO. DEFECATIONS ±SD	TOTAL DEFECATION (ANOVA)	MEAN DEFECATION/2 H
Positive control (Senokot)	68.7 ±1.7	68.66	5.72
Negative control	40.3 ±1.7	40.33	3.36
Ethyl acetate (water insoluble fraction)	39.0 ±1.0	39.00*	3.25*
Ethyl acetate (water soluble fraction)	61.0 ±6.0	61.00**	5.08**
Methanol extract	59.3 ±7.7	59.33**	4.94**

*Values not significant ($P < 0.05$), **Values significant ($P < 0.05$).

TABLE 3. Bacterial cell growth inhibition zone diameter (in mm) obtained with study extracts vs. commercial antibiotic discs.

SUBSTANCE	<i>Escherichia</i>	<i>Staphylococcus</i>	<i>Bacillus</i>
	<i>coli</i> GRAM (-)	<i>aureus</i> GRAM (+)	sp. GRAM (+)
Ether extract	—	—	—
Benzene extract	—	—	—
Chloroform extract	—	—	8
Ethyl acetate extract (water insoluble fraction)	—	—	12
Ethyl acetate extract (water soluble fraction)	22	24	24
Methanol extract	—	—	18
Chloramphenicol (30µg)	34	28	22
Gentamycin (10µg)	20	22	20
Penicillin (10 IU)	10	32	—

discs. Negative control assays of growth alteration due to extract solvents were negative. All extracts, except ether and benzene, produced significant ($P>0.05$) growth inhibition of *Bacillus* sp. No differences were observed in halo size between the methanol extract and water soluble form of the ethyl acetate extract vs. chloramphenicol and gentamycin commercial discs.

DISCUSSION

Analysis of the Bornträger test for anthraquinones, determined in *Senna spectabilis* polar solvent extracts, indicates the absence of other potential compounds that may increase intestinal motility and produce similar *in-vivo* laxative activity. Thus, quinone compounds appear responsible for the effect upon the intestine, and explain the laxative effect obtained by folk medicine using *Senna spectabilis*. Our results are in agreement with reports for other *Cassia* species (Alemayehu *et al.* 1988, Muller *et al.* 1989, Cano *et al.* 1990, and Kitanaka and Takido 1992). The antibacterial activity of *S. spectabilis* extracts (except ether and benzene 1) on *Bacillus* sp. colony growth is comparable to that of commercial discs, and indicates an interesting antimicrobial potential against other sporogenous strains of clinical and industrial importance.

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